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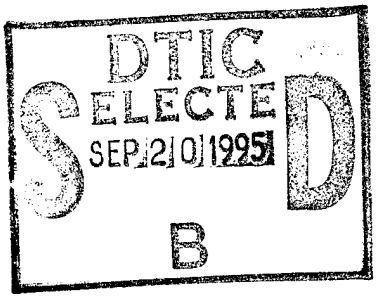

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Nausea and vomiting are severe side-effects often associated with cancer chemotherapy and may affect treatment decisions. Cyclophosphamide is a commonly used chemotherapy agent for breast cancer and induces emesis in the ferret. In order to examine the effects of electroacupuncture (EA) on the emetogenic effect of cyclophosphamide, ferrets (1.0-1.8 kg) were placed under general anesthesia (isoflurane 5%-oxygen mixture) and were administered logarithmic doses of i.v. cyclophosphamide. A dose of 177mg/kg produced the maximal number of emetic episodes (23.3±4.0 episodes) with an emetic profile consisting of two phases (first phase 18.6±3.9 episodes; second phase 4.7±1.2 episodes). For treatment, EA was given under general anesthesia followed by i.v. cyclophosphamide (177mg/kg). Various parameters were evaluated and the results indicated that EA (100Hz, 1.5V, 10 min) effectively treated the first emetic phase induced by cyclophosphamide (9.3±1.8 episodes for first phase). EA had an effect similar to the antiemetic drug ondansetron which also treated the first phase. Preliminary studies using combination therapy of EA and metoclopramide (2.24mg/kg) showed a significant reduction in the number of emetic episodes (p=0.005). This indicates that EA would be useful as an adjunctive therapy for chemotherapy-inducedemesis

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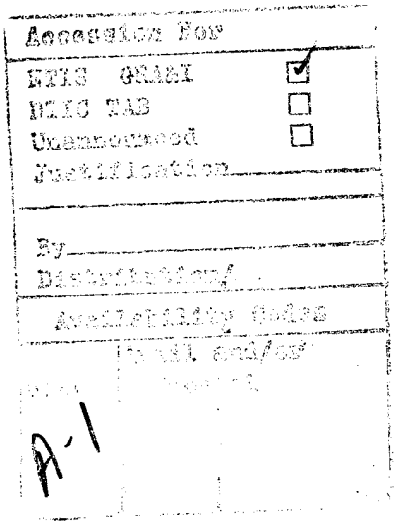

L. Lao 7/27/95
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ANNUAL REPORT

INTRODUCTION

Nausea and vomiting (N/V) are common incidences among patients who have cancer chemotherapy (Coates et al., 1983; Watcha & White, 1992). Antiemetic drugs do not completely block N/V and most often add to the unpleasant effects of treatment (Cubeddu et al., 1990b; D'Olimpia et al., 1985; Watcha & White, 1992). Among the various treatment modalities to reduce N/V, the effect of acupuncture point P6 has been investigated in clinical trials (Aglietti et al., 1990; Dundee, 1991). The clinical studies by Dundee's group indicated that invasive acupuncture combined with antiemetic drug therapy benefited cancer patients in chemotherapy which included cyclophosphamide (Dundee et al., 1989). Other studies conducted by Dundee's group showed that acupressure and transcutaneous electrical stimulation (TENS) of the same acupoints also benefited the patient undergoing chemotherapy (Dundee & Yang, 1990; Dundee et al., 1991). Aglietti's group demonstrated that acupuncture effectively decreased N/V in patients treated with cisplatin (Aglietti, et al., 1990).

Cyclophosphamide is a commonly used agent in chemotherapy for breast cancer and induces emesis in a ferret model (Andrews et al., 1988; Hawthorn et al., 1988). Cyclophosphamide may induce emesis through release of serotonin to stimulate the 5-HT_3 receptor in the gastrointestinal tract and the chemoreceptor trigger zone (Fraschini et al., 1991; Hawthorn et al., 1988). The 5-HT_3 antagonists such as ondansetron have been shown to be moderately effective antiemetics for cyclophosphamide-induced emesis in ferrets (Andrews et al., 1988) and humans (Clavel et al., 1993; Cubeddu et al., 1990a; Fraschini et al., 1991; Rosso et al., 1991). Side effects have included headache, light-headedness and transient elevations of hepatic transaminases (Clavel et al., 1993; Cubeddu et al., 1990a; Einhorn et al., 1990; Fraschini et al., 1991; Hesketh & Gandara, 1991; Rosso et al., 1991). The combination dopamine/5-HT_3 antagonist metoclopramide has been moderately effective in reducing cyclophosphamide-induced emesis in humans (Clavel et al., 1993). Metoclopramide has been shown to produce adverse extrapyramidal side effects in humans (Sanger, 1990). There is no animal model to study the antiemetic effects of acupuncture, however, our pilot study showed that EA given at acupuncture point P6 reduced morphine-induced emesis by 39-43% (Lao et al., 1995).

Acupuncture has been used to treat a variety of diseases, including pain, in China for thousands of years. According to Traditional Chinese Medicine (TCM), there are 12 primary channels or meridians and 8 additional meridians, each following a directional course along the body. A vital energy known as *Qi* flows through these meridians and participates in the homeostatic regulation of various body functions. Some 360 points distributed along the meridians serve as both pathognomic signs of disorder and as loci for acupuncture treatments (O'Connor & Bensky, 1981; Stux & Pomeranz, 1987). The meridian flow of *Qi* can be affected by five climatic factors (heat, cold, damp, dryness and wind) which play a role in the pathogenesis of imbalances resulting in various symptoms or syndromes. Accordingly,

acupuncture treatment involves the insertion of small-gauge needles into specific points as indicated by the nature of the imbalance in order to restore the vital flow of energy through affected meridians (O'Connor & Bensky, 1981; Stux & Pomeranz, 1987). The needles are typically left in place for 20-30 minutes. The effects of acupuncture may be augmented with electrical stimulation (EA) and/or heat (e.g. moxibustion). Side-effects from acupuncture are rare and tend to be associated with violations of sterile procedure and/or negligence on the part of the acupuncturist (Kent & Brondum, 1988; Wright et al., 1991).

A pilot study in our laboratories has shown that the acupuncture technique can be transferred to the ferret by modification of the acupuncture points in humans (Lao et al., 1995). This study showed that EA significantly reduced the number of emetic episodes induced by morphine (Lao et al., 1995). In humans, the acupuncture point Neiguan (P6) is located on the forearm, 2 units directly above the midpoint of the transverse crease of the wrist (the distance between cubital and carpal creases is 12 units), between the tendons of the flexor carpi radialis and the palmaris longus muscles. Below this point is the median nerve (O'Connor & Bensky, 1981). The equivalent point in ferrets had been located in our pilot study (Lao et al., 1995).

The specific aims of the present study are:

1. To determine the emetogenic effect of cyclophosphamide in the ferret.
2. To determine the most effective EA conditions and to evaluate the effect of EA in reducing cyclophosphamide-induced emesis in the ferret.
3. To test the antiemetic effects of ondansetron, metoclopramide, and droperidol against cyclophosphamide-induced emesis in the ferret and to compare these effects to EA.
4. To evaluate that an EA-drug combination is more efficacious as an antiemetic against cyclophosphamide in the ferret than either treatment alone.

BODY

Ferrets were castrated males, 1.0-2.0 kg in weight and from the Triple F Farm, Sayre, PA. Ferrets were made unconscious by general anesthesia (Isoflurane 5%-O_2 mixture) to restrain them for acupuncture treatment. For testing, ferrets were anesthetized with isoflurane 5%-O_2 mixture while contained in a 20 gallon glass aquarium box with a removable plastic cover. The anesthetic gas was delivered from a vaporizer (Fortec), calibrated for isoflurane, through polyethylene tubing into the box and was scavenged out using a vacuum tubing vented to the outside air. Each ferret was removed after loss of righting (2-5 min) and immediately weighed. For EA, each animal was maintained under isoflurane 2.5%-O_2 anesthesia delivered from a second vaporizer through a small nose cone. For EA treatment, the equivalent acupuncture point P6 in the ferret was located at the forepaws (Lao et al., 1995). After needle insertion (disposable needle, gauge # 34, diameter 0.22 mm, length 1 in., depth of 0.3-0.5 in.), the stimulator's electrodes (Grass) were attached to the end of the needles and electrical stimulation was applied (the EA parameters will be described in detail later). The frequency and voltage of stimulation were monitored by an oscilloscope (Tektronix).

Specific Aim #1.

Ferrets were given i.v. cyclophosphamide at log doses of 56, 100, 177, and 237 mg/kg (n=6 for all doses except 237 mg/kg where n=2). For the i.v. route of administration, cyclophosphamide injections were made into the cephalic vein on the dorsal aspect of a front paw using a rubber tourniquet and a 3 or 5 ml syringe with a 25 G needle while the ferret was under general anesthesia. The forepaw was shaved for ease of vein location. Intravenous puncture was confirmed by aspirating a small volume of blood into the syringe and injections confirmed by lack of resistance to the syringe plunger. After injection, each ferret was placed into an individual compartment (60 x 60 x 38 cm^2) of a cage rack holding six compartments having wire mesh floors elevated to the height of door threshold and modified with a plexiglass front door for ease of viewing. Complete recovery from anesthesia occured in all ferrets within 3-10 min. Emetic action of the animal was observed and the onset time of emesis was recorded. The number of episodes of retching and vomiting were also recorded along with the prodromal signs of nausea: salivation, head shake, lip lick, walking backwards, posturing, sedation, and slit eyes (Wynn et al., 1993). Statistical analysis was done using Student's two-way t-test with a $p \leq 0.05$ considered signifcant. After each experiment, the ferrets were sacrificed using carbon dioxide (CO_2). This study (amended 11/29/93) has been approved by the Institutional Animal Care and Use Committees (Ref. #134200-039301 and #93-04-01) at the School of Medicine and the Dental School, University of Maryland at Baltimore.

The results indicated that the dose of 177 mg/kg produced the maximal number of emetic episodes (23.3 ± 4.0 emetic episodes). The dose of 237 mg/kg was not chosen for further experiments since it had toxic effects (Wong et al., 1995a) (Appendix I, Table 1). Cyclophosphamide induced emesis in a dose-dependent manner producing two distinct emetic phases that were separated by a one hour time period (Wong et al., 1995a) (Appendix II, Fig. 1). The first phase resulted in a mean of 18.6 ± 3.9 emetic episodes and the second phase produced 4.7 ± 1.2 emetic episodes. These two phases were used to compare the effects of EA.

Specific Aim #2.

Evaluation of the different parameters of EA were completed (n=6/group). Our results indicated that EA at 100 Hz, 1.5V, 10 min produced the most beneficial antiemetic effect as compared to other parameters . EA was administered followed immediately by i.v. cyclophosphamide (177 mg/kg). EA resulted in a mean of 22.3 ± 3.4 emetic episodes. Although there was no significant reduction in emetic episodes as compared to control, EA was shown to be more effective against the first emetic phase. The results indicated a 50% (mean of 9.3 ± 1.8 emetic episodes) decrease in emetic episodes for the first phase (Appendix II, Fig. 2). Other parameters were also tested (eg. frequency 5 Hz, intensity 3V, and duration 20 min) but did not produce a more beneficial effect (Appendix I, Table 2).

After the determination of the EA parameters, controlled experiments were done to compare the effectiveness of EA (100Hz, 1.5V, 10 min) against cyclophosphamide-induced emesis with respect to sham acupuncture and non-treatment. In the sham acupuncture group, a non-acupuncture point (dummy point) was used at the elbow area (Dundee, 1991), and no electrical stimulation was applied. The results indicated a mean of 25.2 ± 3.8 emetic episodes (14.0 ± 2.7 for first emetic phase; Appendix I, Table 2). In the non-treatment group, animals received the same protocol as the acupuncture group except that the acupuncture needles were taped on the skin of the animal (rather than inserted) and did not have electrical stimulation. This resulted in a mean of 25.0 ± 4.6 emetic episodes (12.2 ± 3.0 for first emetic phase; Appendix I, Table 2). The results showed that EA was able to effectively treat the first phase of emesis induced by cyclophosphamide compared to sham and placebo.

Specific Aim #3.

Studies were done examining the effect of three antiemetic drugs for the treatment of cyclophosphamide-induced emesis: ondansetron, metoclopramide, and droperidol. Using log doses, the antiemetic drugs were administered i.v. immediately following cyclophosphamide injection (177 mg/kg). Ondansetron reduced emetic episodes by 0, 43, and 9% (0.04, 0.07, and 0.13 mg/kg) (Wong et al., 1995a). This drug produced an emetic profile similar to acupuncture in which it was able to effectively treat the first phase of emesis but increased the number of episodes in the second phase (Appendix II, Fig. 3, 4, 5). Metoclopramide reduced emetic episodes by 48, 65, and 98% (2.24, 4.08, 7.07 mg/kg) (Wong et al., 1995a) in which both phases of emesis were reduced significantly at the higher doses ($p \leq 0.05$ at 4.08 mg/kg; $p \leq 0.005$ at 7.07 mg/kg; Appendix II, Fig. 6, 7, 8). However, side effects were also noted at these higher doses and are being analyzed. Droperidol resulted in a 24, 16, and 38% reduction (0.25, 0.45, 0.79 mg/kg) (Wong et al., 1995a)in which it was not able to significantly reduce either phase (Appendix II, Fig. 9, 10, 11).

Specific Aim #4.

Preliminary studies using combination therapy were done in which ferrets ($n=6$) were first treated with EA (100 Hz, 1.5V, 10 min) since it effectively treated the first emetic phase. This was followed by injection with cyclophosphamide (177 mg/kg). The antiemetic drug metoclopramide (2.24 mg/kg) was then given i.v. immediately following cyclophosphamide. Metoclopramide was chosen because it was able to effectively treat the second emetic phase. The results indicated a mean of 6.0 ± 2.1 emetic episodes (Appendix II, Fig. 12) (Wong et al., 1995b). As compared to drug alone (12.0 ± 4.4), this resulted in a 50% decrease in emetic episodes in which the first emetic phase was almost completely eliminated. With respect to control (23.3 ± 4.0), this combined therapy produced a 74% reduction in emetic episodes ($p \leq 0.005$) (Wong et al., 1995b).

CONCLUSIONS

The present study has shown that EA (100 Hz, 1.5V, 10 min) can effectively treat the first emetic phase induced by cyclophosphamide. It has an effect similar to the antiemetic drug ondansetron which also treats the first phase (increases the second phase). Preliminary studies using combination therapy of EA and metoclopramide (low dose) has shown a significant reduction in the number of emetic episodes ($p \leq 0.005$). Drug alone did not produce a significant reduction. This indicates that EA would be useful as an adjunctive therapy in the treatment of chemotherapy-induced emesis. The results also led to a decrease in the variables evaluated (the number of parameters of EA tested and the number of doses of antiemetic drugs used were sufficient) which decreased the number of animals used for this protocol. The next steps in this research are to evaluate the combination of EA with various antiemetic drugs at different dosages. Acupuncture alone will also be examined alone to observe if there are any adverse effects associated with this type of treatment. The significance of this study is that acupuncture as an adjunctive therapy may lead to a decrease in the dose and side effects of the antiemetic drugs which may improve the quality of life for the breast cancer patient. Future clinical studies are necessary to evaluate acupuncture as an adjunctive therapy for the treatment of nausea and vomiting in the breast cancer patient.

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APPENDIX I

Table 1. Dose-Response of Cyclophosphamide-Induced Emesis in the Ferret

Dose of Cyclophosphamide (mg/kg)	Mean No. Emetic Episodes±S.E.
56	2.2±0.9
100	7.3±3.2
177	23.3±4.0
237	23.5±7.5

Note: N=6/group except for the dose of 237 mg/kg (N=2).

Table 2. Effect of EA Parameters, Sham and Placebo Acupuncture on Cyclophosphamide-Induced Emesis

EA Parameters			**Mean±S.E. of Emetic Episodes**
Frequency	*Intensity*	*Duration*	
5Hz	3V	10 min	26.7±3.1
100Hz	1.5V	20 min	27.8±5.4
100Hz	1.5V	5 min	23.5±5.6
100Hz	3.0V	10 min	23.0±7.5
100Hz	1.5V	10 min	22.3±3.4
Sham			25.2±3.8
Placebo			25.0±4.6

APPENDIX II

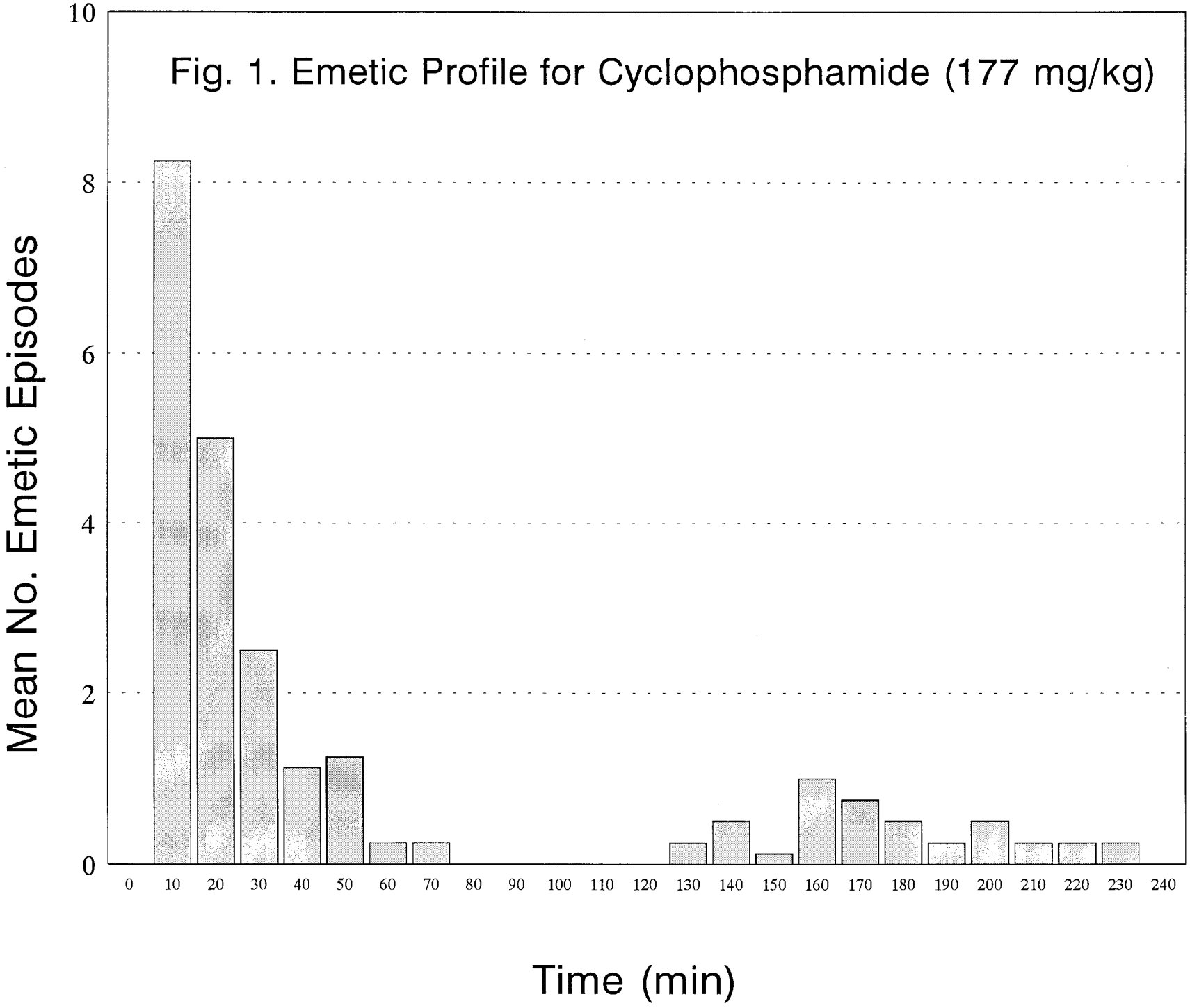
Fig. 1. Emetic Profile for Cyclophosphamide (177 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

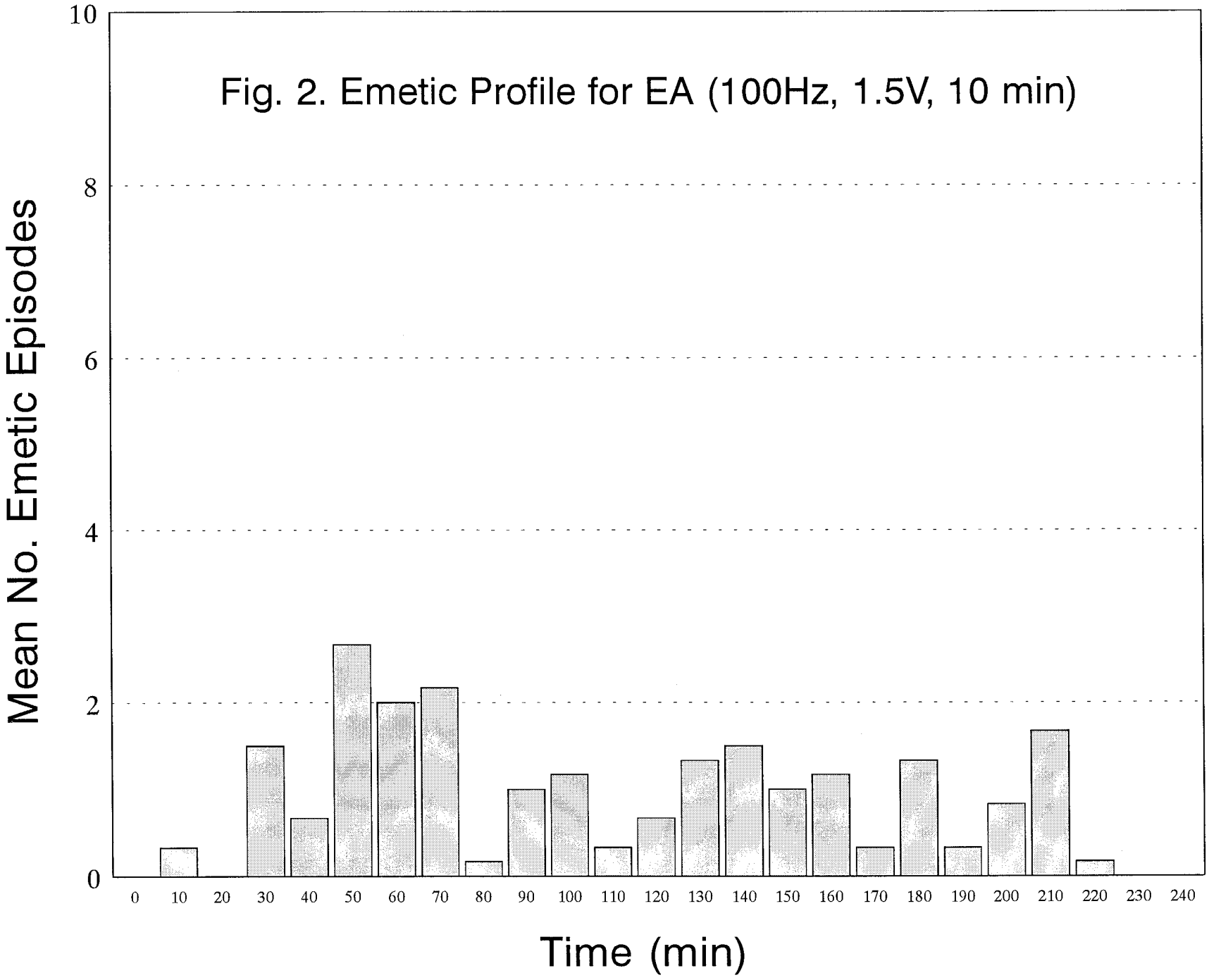
Fig. 2. Emetic Profile for EA (100Hz, 1.5V, 10 min)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

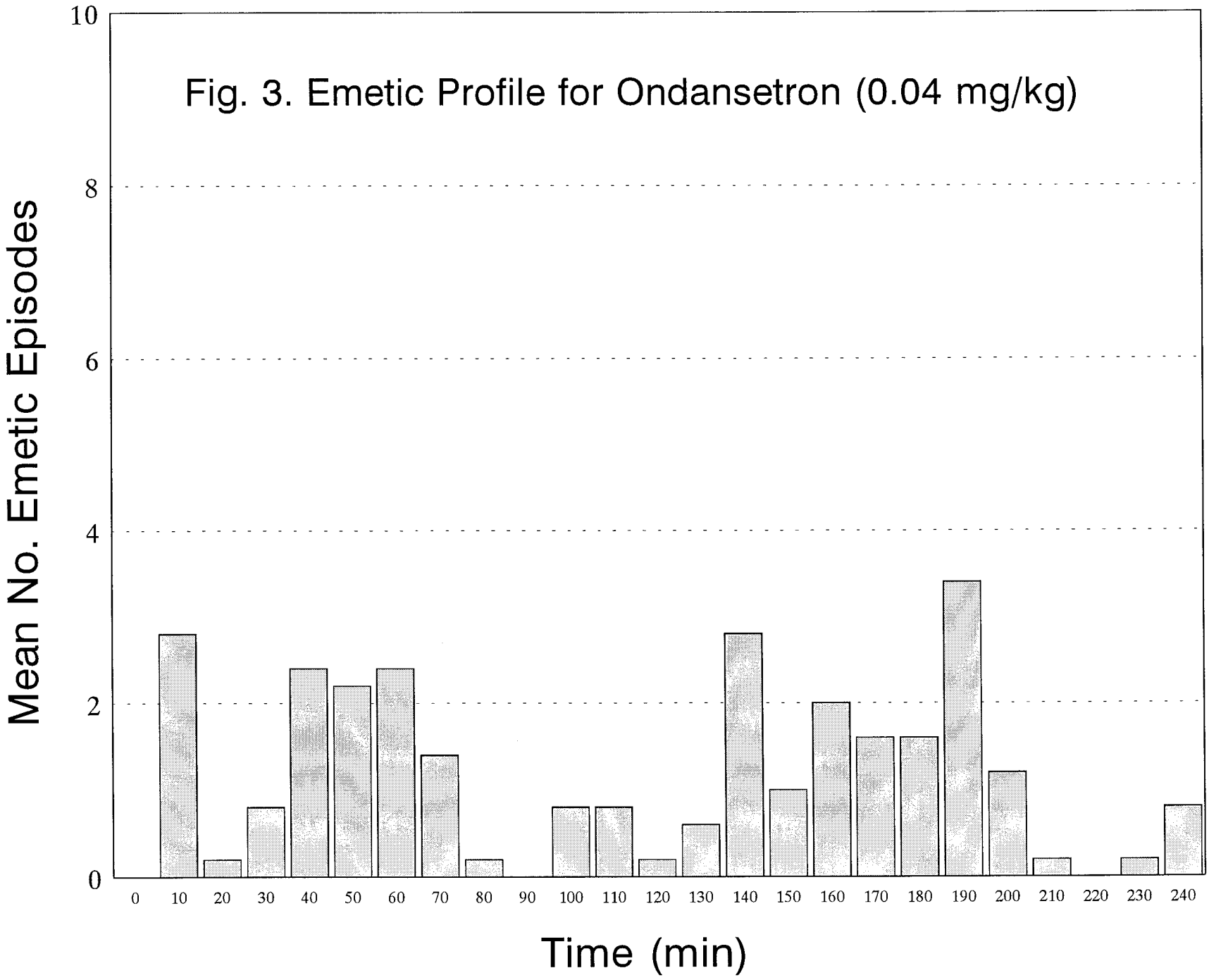
Fig. 3. Emetic Profile for Ondansetron (0.04 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

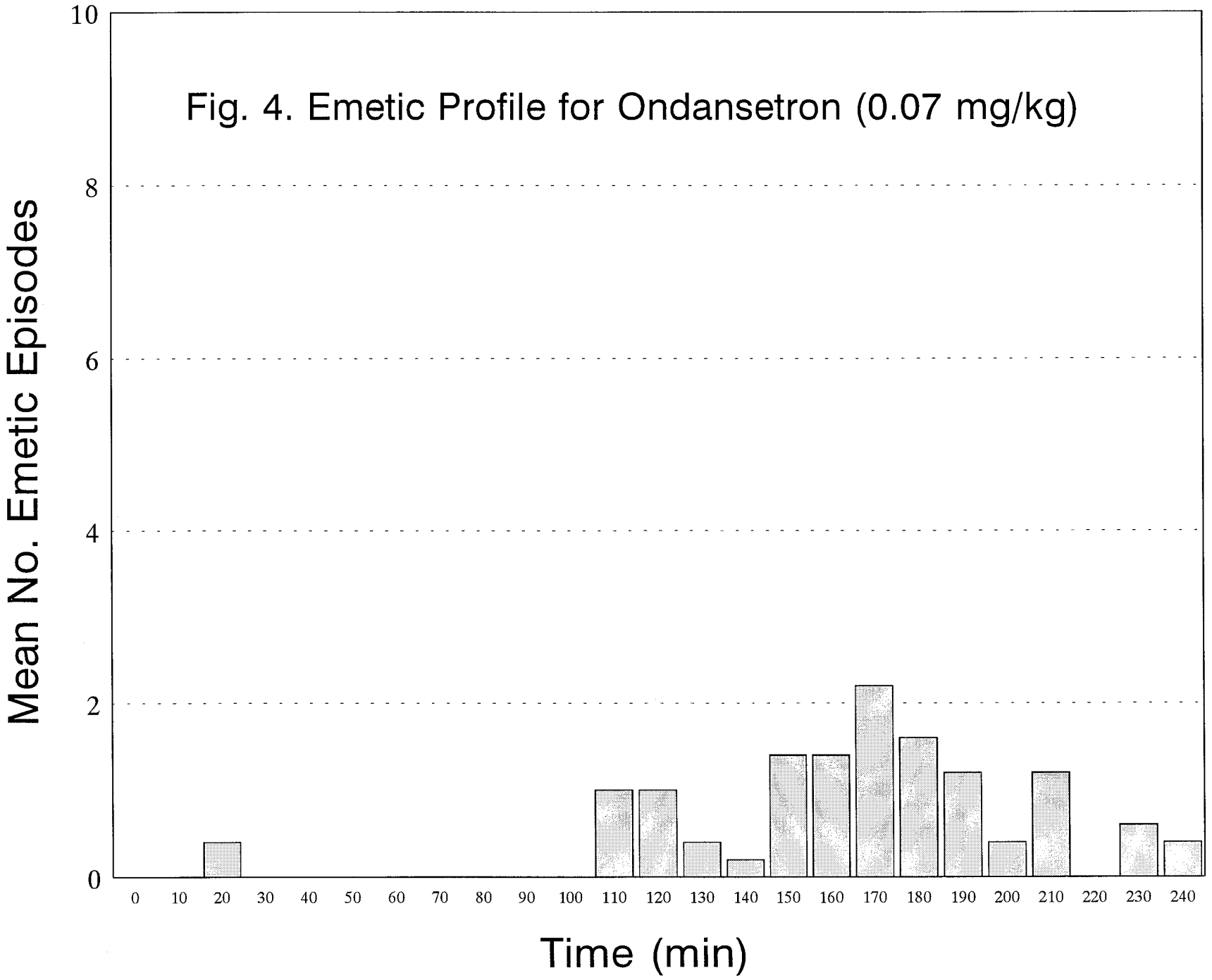
Fig. 4. Emetic Profile for Ondansetron (0.07 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

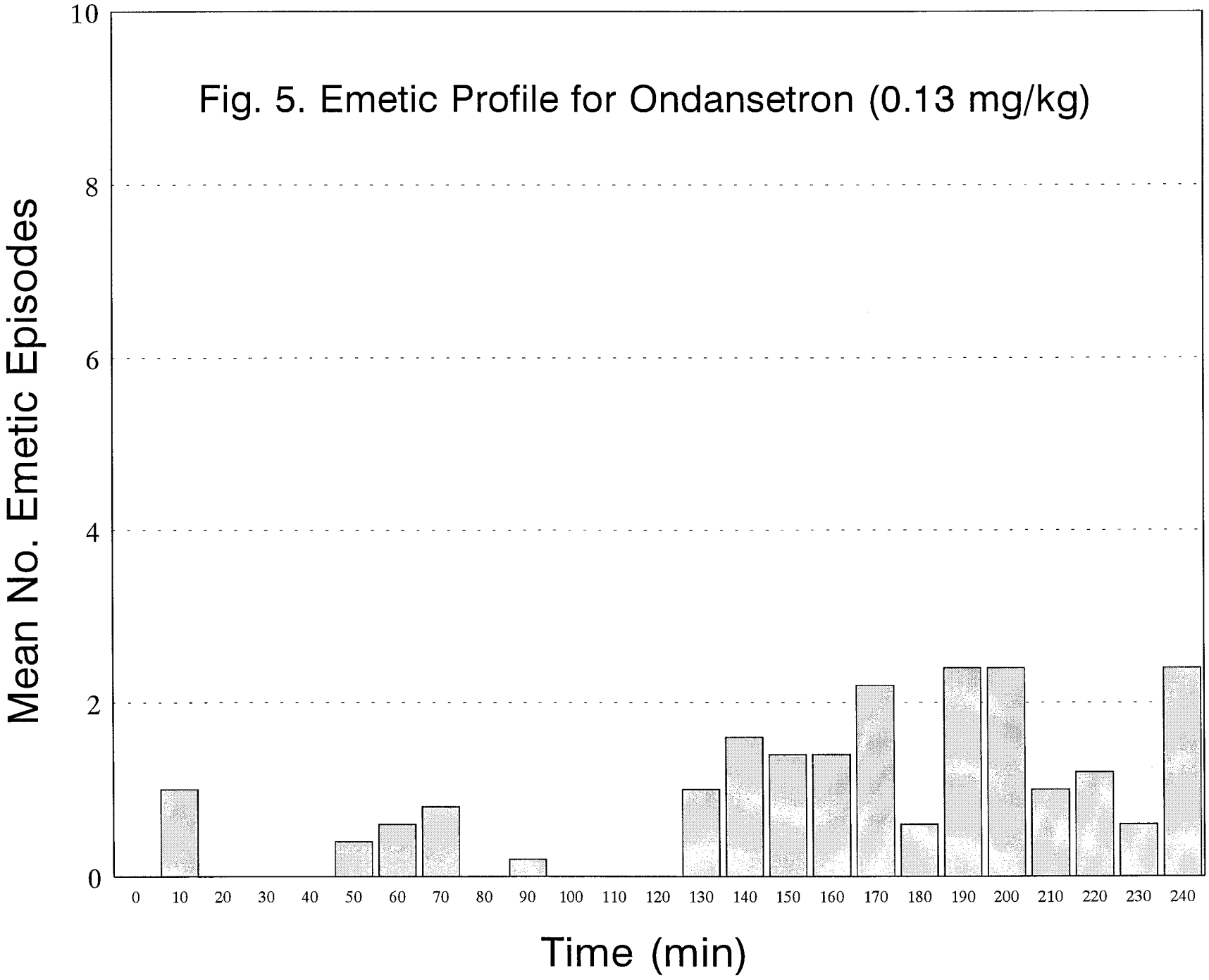
Fig. 5. Emetic Profile for Ondansetron (0.13 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

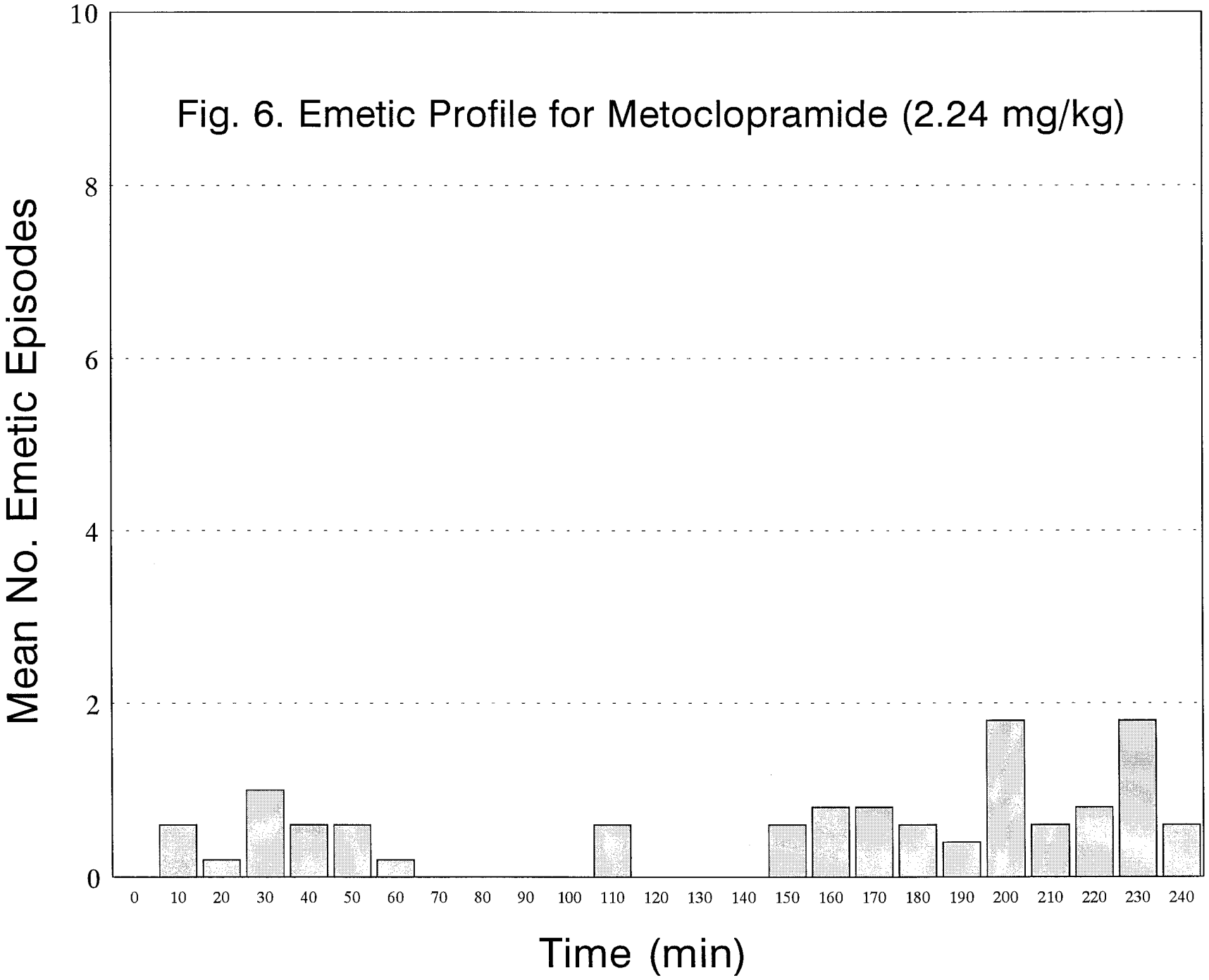
Fig. 6. Emetic Profile for Metoclopramide (2.24 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

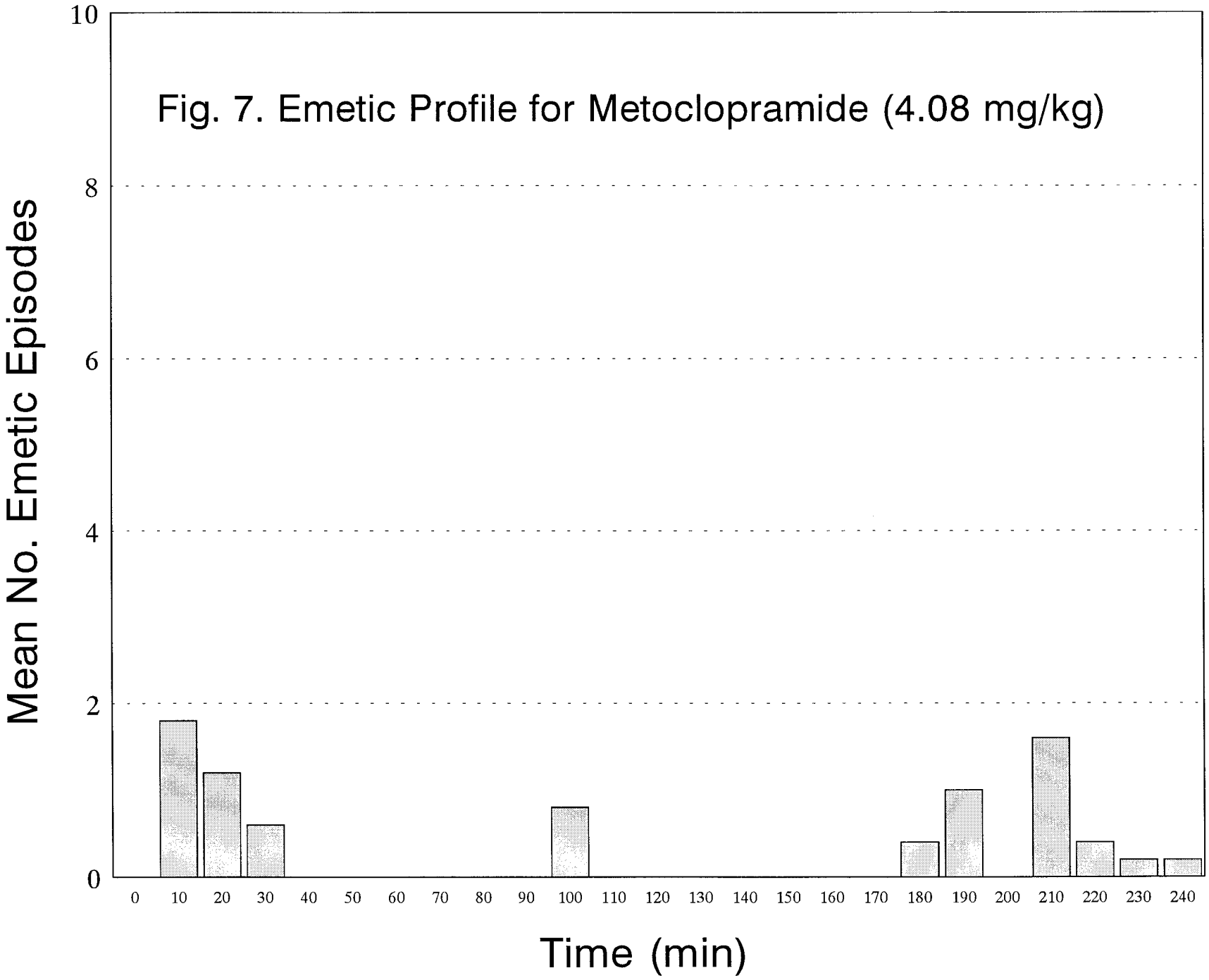
Fig. 7. Emetic Profile for Metoclopramide (4.08 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

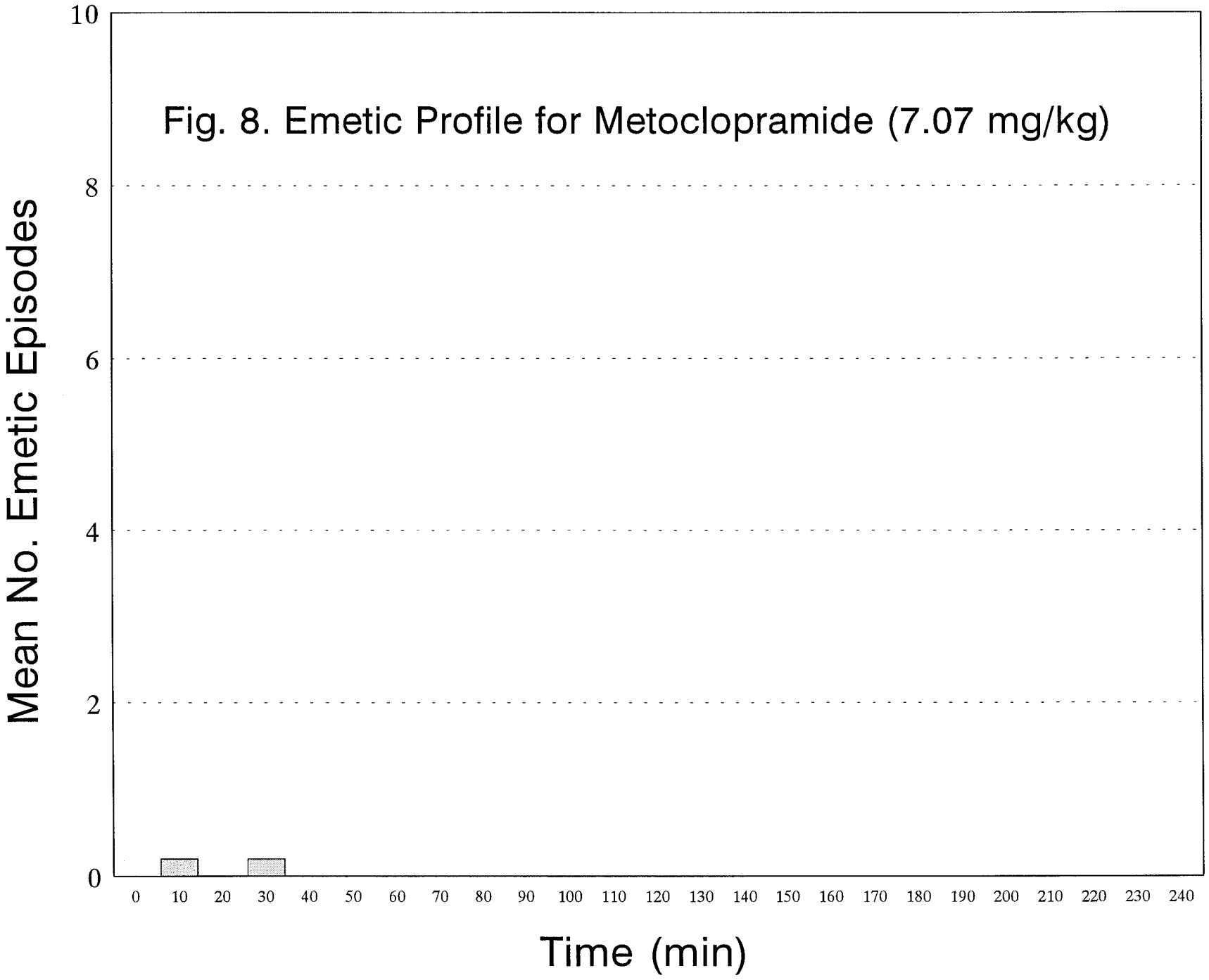
Fig. 8. Emetic Profile for Metoclopramide (7.07 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

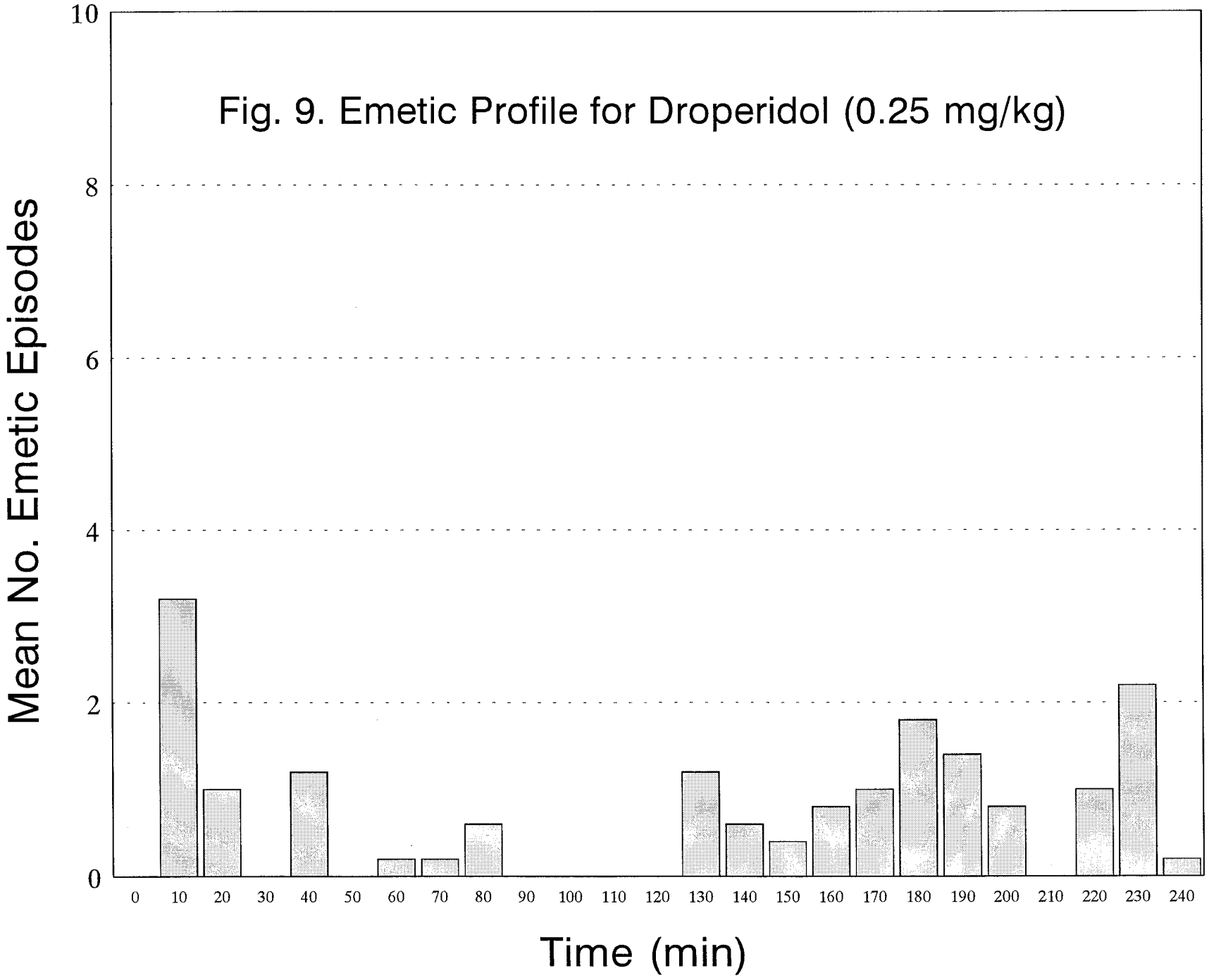
Fig. 9. Emetic Profile for Droperidol (0.25 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

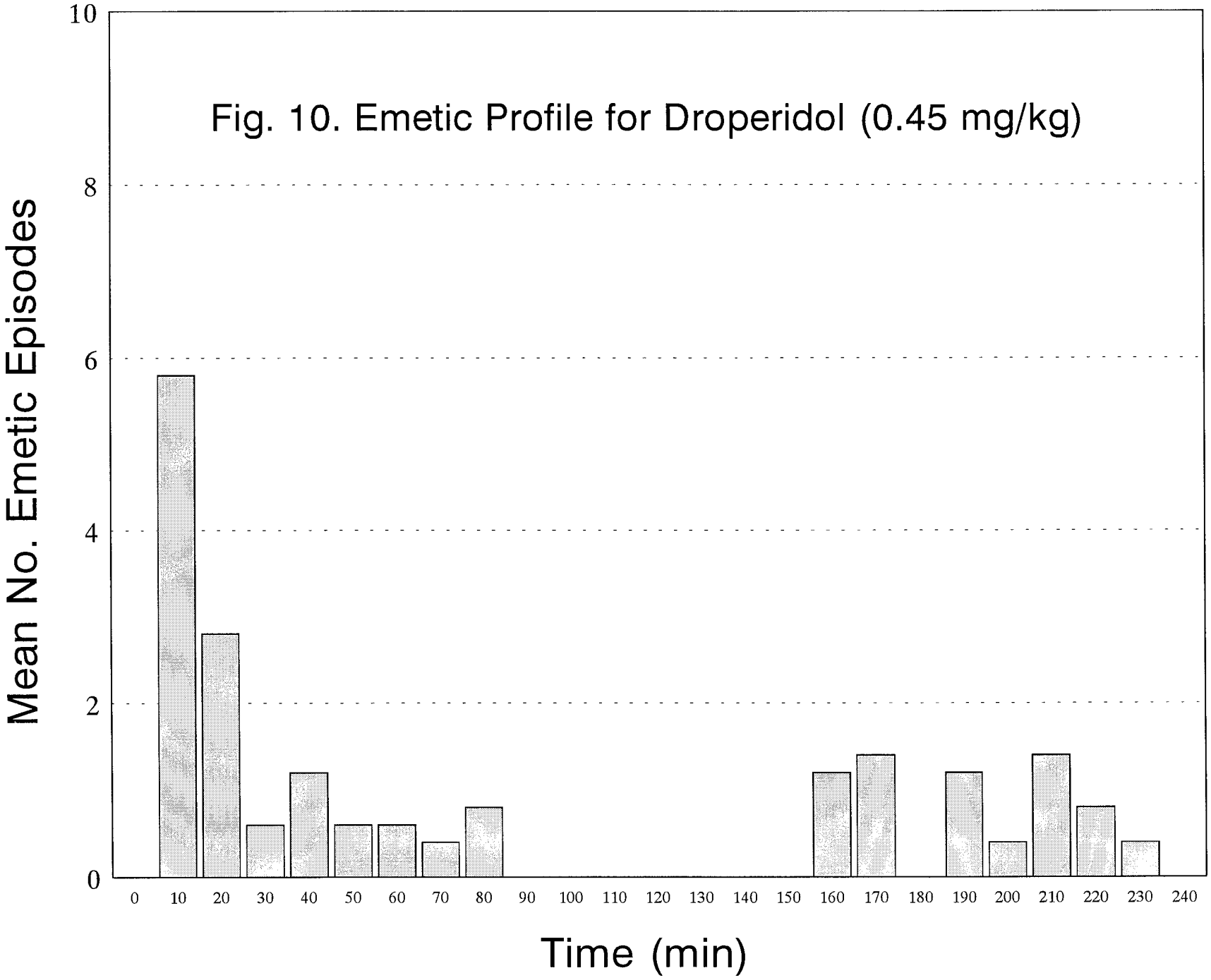
Fig. 10. Emetic Profile for Droperidol (0.45 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

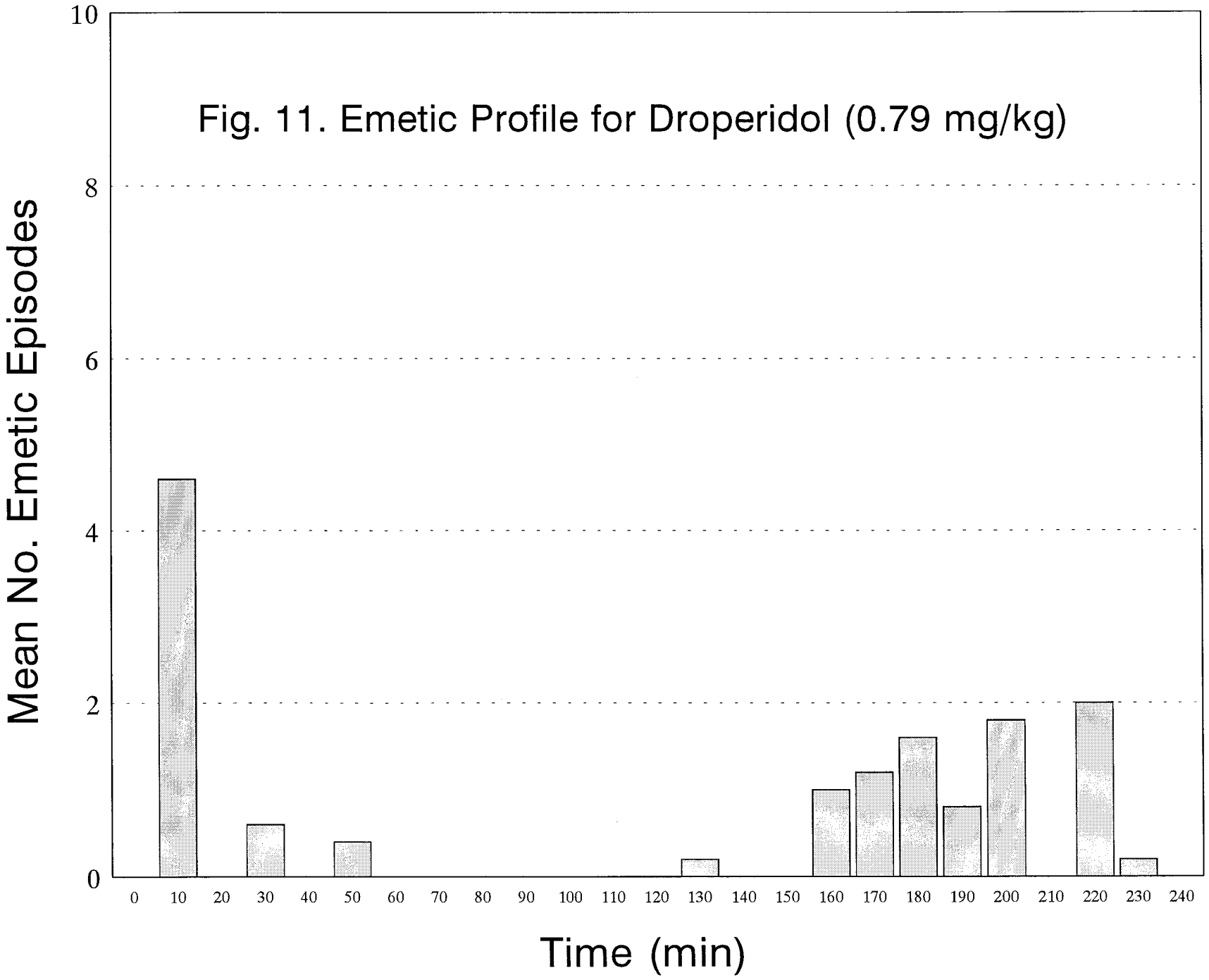
Fig. 11. Emetic Profile for Droperidol (0.79 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)